UPON THE FORMATION OF HYDROCHLORIC ACID IN THE FOVEOLÆ AND ON THE SURFACE OF THE GASTRIC MUCOUS MEMBRANE AND THE NON-ACID CHARACTER OF THE CONTENTS OF GLAND CELLS AND LUMINA.[1]

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CONTENTS.

INTRODUCTION.

Ever since it was discovered that hydrochloric acid was produced by the stomach, investigators have been interested in determining the mode and place of its formation. Is this acid produced as such by the gastric glands and even by particular cells of these glands, or do the glands produce only chemical substances which are not themselves acid but which, interacting in the foveolæ or on the surface of the mucous membrane, produce there for the first time the acid as such?

Among those who have sought to discover the origin of this acid, Claude Bernard, Brücke, Lepine, Trinkler, Gmelin and Oppel were unable to find it definitely localized in the glands.

The results of our investigation demonstrate that only non-acid substances are formed by the glands, and that the contents of the gland cells and lumina are not acid, even when hydro-

[1] From the Hull Laboratory of Anatomy, University of Chicago.

chloric acid is being actively formed on the surface of the mucous membrane.

In the first part of this paper we shall examine very briefly the evidence which has led some to think that free hydrochloric acid is formed in the parietal cells. We shall consider especially the Prussian blue reaction, which, as used by Miss Fitzgerald ('10), has given by far the most definite results. We shall report some results which we have obtained by this method, which in connection with those reported by her show that this reaction does not prove the presence of free hydrochloric acid in the gastric glands under normal conditions.

In the second part of the paper we shall show that the contents of the parietal cells and of the lumina of the gastric glands are not acid but neutral or alkaline, even when hydrochloric acid is being actively formed on the surface of the mucous membrane.

FACTS WHICH HAVE SUGGESTED SOME ASSOCIATION BETWEEN THE PARIETAL CELLS AND THE HYDROCHLORIC ACID OR ITS ANTECEDENTS.

Heidenhain ('70), Langley ('81) and others were able to associate other secretory functions of the gastric mucous membrane very definitely with other cells. This left the parietal cells and the formation of hydrochloric acid. Further, Miss Greenwood ('85), and afterward Macallum ('08) and Miss Fitzgerald ('10), have shown that chlorides are more abundant in the parietal cells than in other parts of the gland. These facts suggest that there is some association between the formation of the hydrochloric acid and the parietal cells. They leave the question quite open, however, as to where the free hydrochloric acid is first formed as such. The parietal cells probably form substances which later furnish the chlorine of the hydrochloric acid, but our results appearing in the second part of this paper show that these cells do not normally contain the hydrochloric acid itself.

Miss Fitzgerald ('10) employed the Prussian blue reaction and obtained very definite results which must be carefully considered in the decision of this question. She found the Prussian blue deposited in the canaliculi of some parietal cells. It will be worth while to examine this reaction and the varying results which have been obtained with it.

The Prussian Blue Reaction.

This was first employed by Claude Bernard ('59) in his classic experiment. A translation of his account is as follows: "In a rabbit which had eaten very little there was injected into the jugular vein a solution of lactate of iron and then a solution of prussiate of potassium; both solutions were warm. Three quarters of an hour afterward the animal was killed and at the autopsy it was impossible to demonstrate the blue color in the tissue of any organ. The urine, which was alkaline and cloudy, was not blue, although it contained both prussiate of potassium and the iron lactate, for it sufficed to add a few drops of hydrochloric or sulphuric acid to cause the blue color of Prussian blue to appear immediately. Upon opening the alimentary canal a blue color was found on the surface of the mucous membrane of the stomach and particularly on the part which corresponded to the lesser curvature of that organ. But this blue was quite superficial; the little deposits of Prussian blue were only on the surface of the mucous membrane, and a microscopic examination did not reveal any Prussian blue in the gastric glands."

Later, Claude Bernard ('77) said: "The acid of the gastric juice is formed only after the secretion of the juice, the glands secreting a liquid which breaks up into an acid fluid and another product as yet not definitely determined."

The results which we have to report indicate that the decision reached by the great French physiologist is correct.

The Prussian blue reaction was employed also by Lepine ('72) in dogs. He used potassium ferrocyanide with lactate of iron or sulphate of iron. He was unable to obtain Prussian blue in any cells of the gastric gland either by injection, maceration, or by passing the salts through a dialyzing membrane made of the gastric mucous membrane, although by the latter method he did obtain a little blue in a lymphatic space of the connective tissue between the glands. He concluded that the acid was not formed as such within the gland.

Sehrwald ('89) put pieces of the gastric mucous membrane into a solution of lactate of iron for one day and later into a solution of potassium ferricyanide. He believed that in this way he would obtain a deposit of Prussian blue at the seat of

formation of the acid. His results were not definite, although he thought the parietal cells showed more blue than other parts of the mucous membrane. The experiment was repeated by Miss Fitzgerald, who found the results too indefinite to decide the question, and by ourselves with the same outcome.

Fitzgerald's Paper.

Miss Fitzgerald ('10) conducted very careful and elaborate experiments by this method and has obtained by far the most definite results. Her experiments and the results are set forth in the following table, which is copied from her paper:[1]

We would like to direct especial attention to a few facts reported n this table and in her paper. First, the plates accompanying her paper show very clearly the deposit of Prussian blue within the canaliculi and in other parts of some parietal cells and also in the interglandular blood and lymph vessels, and in wandering cells and leucocytes. Second, in some experiments the Prussian blue reaction was not obtained. Third, when it did appear it was obtained in only one part of the stomach, namely, that near the œsophagus and along the lesser curvature. This is not the part which contains parietal cells in greatest abundance. Fourth, even in this region only a few of the parietal cells showed the Prussian blue. Those of the deeper third of the gland tubules, that is, the third farthest from the free surface never contained it; and in that part of the gland tubule where it did appear it was found in only a fraction of the parietal cells, so that altogether she found it in only a small percentage of the total number of parietal cells of the stomach, and this notwithstanding the fact that during some part of the long time the experiments lasted all parts of the mucous membrane must have been in full digestive activity. Fifth, it appeared in other structures as well as in the parietal cells, namely, in the blood vessels, in the connective tissue spaces and lymphatic vessels, in wandering cells, and in leucocytes. Sixth, in two instances (rabbits 5 and 6) it did not appear in the canaliculi of the parietal cells but only at the surface of the latter remote from the lumen and next to the blood vessels.

Our own results have confirmed these facts entirely.

TABLE GIVING RESULTS OBTAINED BY INJECTING A SOLUTION CONTAINING EQUAL PARTS OF AN AQUEOUS SOLUTION OF 2.25 PER CENT. AMMONIUM FERRIC CITRATE AND OF 1.5 PER CENT. POTASSIUM FERROCYANIDE.[1]

Animal.	Mode and No. of Injections.	Amount Injected. C.c.	No. of Hours Since First and Last Injections.		Condition.	Toxic Effect.	Prussian Blue Reaction—Spontaneous.			Ditto on Addition of 0.5 Per Cent. HCl.	
							Gastric Mucosa.		Skin.	Urine.	Various Tissues.[2]
			First.	Last.			Surface.	Interior.			
Rabbit (1)...	Intravenous, 1 Subcutaneous, 2	10–11	$35\frac{1}{4}$	2	Food given.	−	−	−	−	+	+
Rabbit (2)...	Subcutaneous, 8	37	30	$2\frac{1}{2}$	Food given.	−	+	+	+	+ (1st day) − (2d day)	+
Rabbit (3)...	Subcutaneous, 5	31–32	$25\frac{3}{4}$	$2\frac{1}{2}$	Starved 24 hrs.	− (1st day) + (2d day)	+	−	−	− (Retention of urine)	+
Rabbit (4)...	Intravenous, 2	17	$4\frac{1}{2}$	$2\frac{3}{4}$	Semi-fasting. (Starved 36 hrs. Food. Starved 24 hrs.)	−	−	−	−	+	+
Rabbit (5)...	Subcutaneous, 5	50	$6\frac{1}{2}$	1	No food eaten during expt.	+	+	+	−	+	+
Rabbit (6)...	Subcutaneous, 4	43–45	3	$\frac{3}{4}$–1	Food before exp.	−	+	+	−	+	+
Rabbit (7) (sod. ferrocyanide)	Subcutaneous, 4	40	$5\frac{1}{4}$	1	Semi-fasting. Food given after fast of 20 hrs. and during expt. Eaten well until after 4th injection.	+	−	−	−	+	+
Guinea-pig (1)	Subcutaneous, 2	22	$3\frac{3}{4}$	$1\frac{3}{4}$	Food given.	+	+	−	+	+	+
Guinea-pig (2)	Subcutaneous, 2	16	$5\frac{1}{4}$	3	....	+ (Polyuria)	+	−	−	+−?	+
Guinea-pig (3)	Subcutaneous, 2	19	$6\frac{3}{4}$	$4\frac{1}{2}$	....	+ (Polyuria)	+	−	+	+	+
Dog (1).....	Subcutaneous, 2	55	$3\frac{1}{4}$	$1\frac{1}{4}$	Starved 48 hrs. Food given 1 hr. before expt.	+ (Vomiting)	−	−	−	−	+
Dog (2).....	Subcutaneous, 2	42	$3\frac{1}{4}$	..	....	+ (Comatose)	+	−	+	+	+

\+ means positive result. − means negative result.

[1] M. P. Fitzgerald, 1910, *Proceed. Roy. Soc.*, B. 83.

[2] After their removal from the body, and subsequent exposure to the action of dilute hydrochloric acid, the Prussian blue reaction developed, at various times, in the following tissues: Bladder, kidney, liver, small and large intestines, ovary, fallopian tubes, uterus, vagina, and lymph gland of axillary region (dog). The kidney became quite blue in many cases, and under the microscope the uriniferous tubules presented a very striking appearance.

From these results Miss Fitzgerald concludes that the hydrochloric acid was formed as such in the parietal cells and secreted into the gland lumina. She says (p. 82): "The occurrence of the Prussian blue reaction in the canaliculi of the parietal cells of an animal injected with a solution of these two salts affords conclusive evidence of the presence of free acid within these structures."

The absence of the Prussian blue from most of the parietal cells might be regarded as evidence that only a few of these cells are engaged in the formation of hydrochloric acid, and that the greater part of them do not form it. She explains this absence from most of the parietal cells by saying that it may have been washed out of them, but surely in that case it would have appeared in them in some preparations. She explains the appearance of the reaction in the blood vessels and in the part of the parietal cell next to the blood vessel (and that part only) by the suggestion that under certain circumstances the acid may be secreted by the parietal cells into the blood stream instead of into the gland lumen. But the facts she reports are open to the other interpretation that the Prussian blue, or the salts forming it, may have been excreted from the blood stream into the parietal cell.

Our Own Experiments.

These were conducted upon rabbits, cats, dogs, a fowl, a snapping turtle, and several skates. Into these animals we injected solutions of sodium ferrocyanide (which we found less toxic than potassium ferrocyanide) and solutions of iron and ammonium citrate. We did not always use molecularly balanced solutions, because we found that the two salts were excreted with very different degrees of rapidity and by different ways. The injections were made subcutaneously or intravenously. In so far as our experiments repeated those of Miss Fitzgerald, they confirmed the results reported by her entirely, but we obtained also some additional results which have a very important bearing upon the conclusion which she drew.

By these experiments we sought to get answers to the following questions: First, is the Prussian blue precipitate produced in any place other than the gastric mucous membrane?

Prussian Blue in Places Other than Gastric Mucous Membrane. —Into the ear vein of a rabbit was injected 13 c.c. of a 10 per cent. solution of sodium ferrocyanide, and into the other ear vein 10 c.c. of a 25 per cent. solution of iron and ammonium citrate; both solutions were warm. They were perfectly fresh, having just been made. They did not give a precipitate of Prussian blue when diluted each with an equal quantity of water, one added to the other, and the mixture allowed to stand in a warm chamber at 37° C. for 28 hours. The rabbit showed some toxic effects during the injection, rallied temporarily, but died in 20 minutes. The stomach was opened at once. It was full of fresh food in an active state of digestion. Prussian blue appeared in all parts of it, although the lesser curvature seemed to have less of it than the rest of the stomach. Pieces of various tissues were fixed in absolute alcohol. The urine showed both salts present in abundance; the bile contained none of either. The saliva contained ferric citrate but no ferrocyanide. The heart's blood showed no blue on the addition of acid nor on the addition of acid with each one of the two salts. Therefore, it did not contain either salt in any quantity appreciable by this method. Paraffine sections were cut and they showed the Prussian blue reaction on the surface of the stomach; in the mucous membrane of the stomach appearing in the blood vessels, in the lymphatic spaces, in the epithelium between the foveolæ; in the connective tissue of the muscularis mucosæ and of the tunica muscularis of the stomach. It did not appear in the parietal cells nor in any other gland cells, nor in the gland lumen. It was found in many other tissues, namely: in the liver, appearing in the blood vessels, in the endothelial cells of Kupffer, and a little in the bile capillaries; in the spleen appearing in the blood vessels; in the blood vessels of the heart muscle. These were the only places in which we looked for it, but we found it in all of them.

This experiment was repeated, using smaller quantities of the salts injected subcutaneously on two successive days (a total of 1.6 grammes iron and ammonium citrate and 3.0 grammes sodium ferrocyanide well diluted). The rabbit was killed 30 minutes after the second injection; the tissues were fixed in formalin (neutralized with magnesium carbonate). The Prussian blue

reaction was found in all places in which it was found in the first rabbit. In addition it was found in the villi of the duodenum appearing in the connective tissue near the free end of the villus, and also between the epithelial cells of the free end of the villus. The blood vessels of the gastric mucosa contained Prussian blue, but in much smaller quantities than did those of the spleen and liver. The parietal cells were practically free of it; there may have been a little, for three or four minute particles were found in the section, but it was impossible to say that these extremely minute particles were not on the surface of the cell instead of within it.

From these two experiments it is evident that the Prussian blue is precipitated in the blood stream when solutions of these salts are injected into it. It may also be precipitated or absorbed in various places, especially in the endothelial cells of Kupffer in the liver. They show that it may appear on the surface of the stomach when it is not in the parietal cells; that it appears in the blood vessels and lymphatic spaces before it appears in the parietal cells. Its appearance between the cells of the surface epithelium between adjacent foveolæ, and also in the interglandular blood vessels and lymphatic vessels beneath this epithelium suggests that the Prussian blue or some or all of the salts necessary for its formation may pass from the surface into the interglandular lymphatics. These results taken in conjunction with those reported by Miss Fitzgerald in her rabbits Nos. 5 and 6 suggest very strongly that the Prussian blue or the constituents forming it may pass from the blood vessels into the parietal cells instead of in the reverse direction as she supposed. In other rabbits we have frequently seen the Prussian blue in the blood vessels and in only those parts of a few parietal cells lying next to the blood vessels, just as she reports in her rabbits 5 and 6. We have never found it in the parietal cells without finding it also in the blood vessels, but in these two instances we found it in the blood vessels when it was absent from the parietal cells. When it was present on the surface of the stomach we found it uniformly present also between the cells of the interfoveolar surface epithelium and in the interglandular lymphatic vessels and blood vessels. We have fre-

quently found it similarly in the tips of pyloric and duodenal villi, between the epithelial cells and in the subjacent connective tissue. Similar results were obtained in a fowl which had been given subcutaneously 13 c.c. of a 10 per cent. solution of sodium ferryocyanide and 13 c.c. of a 25 per cent. solution of iron and ammonium citrate, and two hours later intravenously 7 c.c. of the sodium ferrocyanide solution and 4.5 c.c. of the iron and ammonium citrate solution; both solutions were fresh and warm. The animal stood these injections very well, although they had some toxic effect. The fœcal passages were liquid and contained a little blue precipitate which became quite heavy and abundant on the addition of hydrochloric acid. The animal was killed in one hour by chloroform, and pieces of various tissues were fixed in absolute alcohol. When the proventriculus was opened a blue fluid welled out of the openings of the depressions which contain the compound glands; no blue color appeared elsewhere in this organ. Microscopic examination of paraffine sections showed Prussian blue on the surface of the epithelium lining the necks of the depressions of the surface epithelium into which the glands open and in the lumina of these depressions. It was not within the glands themselves. It was present between the epithelial cells of the region where the depression receiving the secretion of the glands opened on to the surface of the proventriculus and in the subjacent blood vessels and lymphatic spaces. It was abundant in the blood vessels and lymphatic spaces of the muscular coat of the stomach, and of the crop, and of the heart muscle, and of the muscular stomach (gizzard). It appeared also in the epithelium of the intestine and in the connective tissue and muscle of the cœcal diverticula. It was abundant in the liver appearing in the endothelial cells of Kupffer and in the blood vessels. It was absent from the breast muscle and its lymphatic and blood vessels.

These results, therefore, answer this first question in the affirmative. The Prussian blue reaction appears in many places besides the gastric mucous membrane. In most of them it seems probable that it takes place without the help of an acid. And they show that, if its occurrence on the surface of the gastric mucous membrane is due to the acid formed in the stomach then

that acid is present on the surface while it is absent from parietal cells and from the gland lumen.

Effects of Injury on Amount of Prussian Blue Precipitate.—Second, is the amount of Prussian blue precipitate increased by mechanical injury of the mucous membrane? A small kitten was given by subcutaneous injection small doses of a mixture of solutions of potassium ferrocyanide and iron and ammonium citrate in molecular proportions. These injections were given three times a day for three days. On the third day the abdomen was opened, a small piece of mucous membrane was removed from the fundus region of the stomach, the wound in the mucous membrane was sutured with silk sutures and afterward the other coats of the stomach were united and the abdomen was closed. The operation was done with aseptic precautions. Five hours later the kitten was killed, pieces of tissue were fixed in alcohol and in neutral formalin (freshly distilled over potassium hydrate). In the immediate vicinity of the suture paraffine sections showed that the Prussion blue reaction occurred in great abundance in the blood vessels, in the lymphatic spaces and in the parietal cells. These preparations showed blue in a very large proportion of the parietal cells and even in those at the bottom of the tubules. It was especially abundant in the parietal cells of the necks of the glands. Many parietal cells were dead and thrown off into the gland lumen; in every instance these dead cells were filled with Prussian blue. In those cells which were still in their normal position many showed the Prussian blue in the canaliculi disposed in a manner very similar to that which Miss Fitzgerald has shown in her Plate VII., Figs. 6 and 8. As one proceeded in the study of these sections progressively farther from the site of injury the amount of Prussian blue and the number of parietal cells showing it progressively decreased, and in some sections of mucous membrane taken from parts of the stomach remote from the site of operation and apparently in a healthy condition, the parietal cells did not contain any blue at all.

In another cat a similar operation was performed on the gastric mucous membrane and during four successive days following solutions of potassium ferrocyanide and of iron and ammonium

citrate in equal quantities were injected subcutaneously. On the fourth day the cat died, but the tissues were immediately fixed in neutral formalin. There was more Prussian blue in the immediate neighborhood of the sutures than in other parts of the mucous membrane.

These results answer the second question in the affirmative. And they show that dead parietal cells show more Prussian blue than living ones. The dead ones are always stained by it, whereas most of the living ones are not. This suggests that the death of the parietal cell or a lowering of its vitality may permit or facilitate the penetration of the cell by substances concerned in the Prussian blue reaction, and that dead cells become acid.

Effect of Poisons on Amount of Prussian Blue Precipitate.—Third, is the extent of the Prussian blue reaction increased by the injection of poisons into the wall of the stomach? We injected into the submucous tissue of the stomach exposed under aseptic precautions, solutions of phosphorus in olive oil, and solutions of moccasin venom in distilled water. The incisions in the abdominal wall were closed and salts of sodium ferrocyanide and iron and ammonium citrate injected subcutaneously for varying periods. The results were negative; we could not demonstrate any increase of the Prussian blue reaction in the area where these poisons were injected.

Effect of Restriction of Blood Supply on Amount of Prussian Blue Precipitate.—Fourth, is the amount of Prussian blue increased in areas of restricted blood supply?

Arteries and veins of various sizes were tied on the stomachs of different rabbits and cats. Solutions of sodium ferrocyanide and iron and ammonium citrate were injected subcutaneously during several days. The results answered this question in the negative. There was no increase of Prussian blue in the areas of restricted blood supply. In a few cases ulcers were produced and on the surface of these there was always a considerable deposit of Prussian blue. The cells on the surface of the ulcer belonging to various parts of the gastric glands and presumably dead or dying always showed a considerable deposit of Prussian blue in them.

Precipitates Do Not Back into Gland Lumina from the Surface.—

Fifth, is the occasional presence of Prussian blue in the gland lumen and in the canaliculi of the parietal cells due to backing up of the blue precipitate from the foveolæ or from the surface of the mucous membrane.?

In order to determine this question many experiments were made with Prussian blue, with carmine and with India ink. Hydrostatic pressure, dialysis, and positive pressure by a syringe piston against a piece of gastric mucous membrane tied over the end of the syringe were employed. The results were all negative, thus confirming those of Lepine ('72).

Prussian Blue Reaction in Animals which Have no Parietal Cells, but Secrete Acid.—Sixth, where does the Prussian blue reaction occur in animals which have no parietal cells but yet secrete acid?

The experiment above reported with the fowl showed that the Prussian blue occurred in the lumen of the depression receiving the secretion of the compound glands of the proventriculus. It was not found in the gland cells. It appeared between the cells of the surface epithelium immediately adjacent to the opening of the depressions. As above stated, it occurred also in many other places.

A snapping turtle was given one half gram each of sodium ferrocyanide and iron and ammonium citrate in dilute solution three times daily during four days; the solutions were given separately and subcutaneously in the inguinal region. Two small fish were shoved into the stomach with a glass rod and were found there later partially digested. On the fourth day the animal was killed and the stomach examined. A deposit of Prussian blue was found in every cell of the somewhat coarse foveolæ of the gastric gland. In these cells it occupied a definite position, the same in each cell. Each cell contained a mucous plug which occupied the half of the cell next the lumen, under it a small spherical mass of Prussian blue, and under that the nucleus. The mass of Prussian blue was nearly as large as the nucleus. Sections through these foveolæ stained with paracarmine or mucicarmine presented a very pretty appearance because of the extreme regularity of the position of the nuclei, the little masses of Prussian blue and the mucous plugs. There

was more Prussian blue in the cells of the foveolar epithelium then anywhere alse. The amount in the cells of the necks of the gland was less and decreased toward the bottom of the gland tubule, although it appeared in nearly all the cells of the gland. It was abundant in the interglandular connective tissue, being in the blood and lymph vessels.

Skates experimented upon in the same way showed Prussian blue in the blood vessels and lymphatics of the wall of the pharynx and stomach. The glands of the gastric mucous membrane showed the reaction in small quantities in the gland lumina and in a few of the cells. The cells showing the reaction were more numerous in the part of the gland near the free surface than in its deeper portions. The surface epithelium of the stomach and of the intestines two inches beyond the pylorus and of the large intestine contained small quantities of Prussian blue.

Summary of the Results Obtained by the Prussian Blue Reaction and their Significance.

From the facts reported by Miss Fitzgerald and those which our own experiments have added, it seems clear that the occurrence of the Prussian blue reaction does not necessarily indicate the formation of free mineral acid under normal conditions in the places in which it is found. It appears in many places where it could hardly have been due to the presence of acid—in the blood, lymph, liver, spleen, intestine, heart muscle, etc. In these places its presence must be due to something else.

It may be due to fatty acids, which bring about the precipitation of Prussian blue from solutions of the salts used in these experiments in proportion to the amount of the acid present. It may be due to the withdrawal of the ammonium citrate by more rapid diffusion or by the involvement of the ammonium in the metabolic processes of the tissues. Nencki and Pawlow ('96) have shown that the gastric mucous membrane normally contains an extraordinarily large amount of ammonia. Ferric citrate and sodium ferrocyanide solutions give when mixed an immediate precipitate of Prussian blue even in the absence of any acid. It may be due to the death or reduced vitality of cells, permitting

the entrance of salts which would not have entered living or healthy cells. It may be that the cells which show the reaction are just those which are poisoned by the salts used. The iron and ammonium citrate solution injected repeatedly during several days caused the death of one kitten. Therefore this salt has a serious toxic action. It may be that the interaction chemically of these two salts with the contents of certain cells may sometimes permit the liberation of an acid in them, when no acid would have been produced in the absence of the complex chemical state which exists when they are present. It may have been absorbed from the surface of the mucous membrane. Its presence between the epithelial cells and in underlying lymphatic vessels of the stomach and intestine suggest this possibility.

Since the Prussian blue may be precipitated in so many places, the fact that it is sometimes precipitated in the canaliculi of a few parietal cells in a relatively small part of the stomach perhaps in an abnormal condition at the time does not necessarily prove that free hydrochloric acid is formed under normal conditions in the parietal cells of the stomach as a whole. The failure to get any reaction in the stomach in some experiments, the small number of parietal cells in which it ever appears, its occurrence in other tissues and in the blood vessels and lymph vessels before it appears in the parietal cells at all, the fact that several factors other than the presence of free mineral acid may cause the precipitate to form,—all these things show that it would not be right to conclude from the evidence which the Prussian blue reaction affords that free hydrochloric acid is formed in the parietal cells. Much less could one reach this conclusion from any other evidence that has been adduced, for all other evidence is much less definite than this. And this failure to show clearly that free hydrochloric acid is formed in the parietal cells becomes quite clear when it appears, as we shall show in the following part of this paper, that the contents of the canaliculi of these cells are alkaline and those of the gland lumina are not acid when free acid is being produced by the mucous membrane.

EXPERIMENTS WITH INDICATORS: THE REACTION OF THE SECRETION WITHIN THE LUMEN OF THE ACTIVE FUNDUS GLAND, AND WITHIN THE INTRACELLULAR CANALICULI OF THE PARIETAL CELLS.

It is obvious from the observations of Fitzgerald and ourselves reported in the preceding section that the Prussian blue reaction is not a trustworthy indication of the place of formation of the hydrochloric acid of the gastric juice, and that we must look to other methods for a solution of this problem. One naturally turns to the chemical indicators for this purpose. The results obtained by means of these substances by previous investigators who have employed them have been uniformly unsatisfactory and unconvincing. The most definite results obtained by these methods are those reported by Fränkel ('91) who used neutral sodium rosanilinsulphonate as an indicator, and by Edinger ('79), who employed a solution of sodium alizarin.

Rosanilin sulphonic acid possesses the property of forming with sodium hydroxide acid salts which form red solutions (acid fuchsin), and neutral salts whose solutions are colorless. The addition of small quantities of acid to solutions of the neutral salt results in the production of the red colored acid salt. Accordingly, Fränkel injected into the jugular veins of dogs 50–100 c.c. of a 5 per cent. solution of the neutral sodium rosanilin sulphonate. As a result of this proceeding he found the entire mucous membrane of the stomach, including the pyloric mucous membrane, stained brilliant red. Teasing portions of the mucous membrane in distilled water he found that both parietal and chief cells were stained in the fundus glands, and that the cells of the pyloric glands were also stained, while the cylindrical cells of the surface were unstained. He could see no difference in the intensity of the stain in the two types of cells. The same experiment was also performed on rabbits, but in this case he found that the color was not uniformly distributed throughout the mucous membrane, and the pyloric mucosa showed only a few slightly red spots.

From these experiments Fränkel concludes: that the mucous membrane of the stomach has an acid reaction; that the acid is formed in the parenchyma cells; and that it can always be

demonstrated in them. Regarding the acid reaction obtained by this method in the pyloric region he does not venture an interpretation.

The results obtained by Edinger by means of the sodium alizarin reaction were similar to those of Fränkel. Sodium alizarin, as Edinger pointed out is, in neutral solutions, of a deep purple red color, while the addition of an acid results in the precipitation of the alizarin as a flocculent yellow precipitate. Edinger prepared the solution by adding alizarin in excess to a 10 per cent. solution of sodium hydroxide. Then the solution was filtered. 25–100 c.c. of this solution were injected into the jugular veins of rabbits and dogs. In a rabbit he found after this injection the stomach spotted red-violet and yellow, the latter being more general in the region of the greater curvature, though the pyloric mucous membrane was also yellow. He concludes that the glands of the rabbit's stomach are not all in activity at the same time, and that both the fundus and pyloric mucous membranes react acid. In dogs, after similar treatment, the whole mucous membrane of the stomach, including that of the pyloric region, was yellow. Sections of the mucous membrane showed that the yellow color was to be found at all levels, but the intensity of the stain was too slight to permit of the recognition of the stain in particular cells. The pancreas also gave an acid reaction.

Experiments with tropeolin, congo red, litmus, phenolphthalein, and other indicators in common use, have been without result or, at the most, have only indicated what was known from examination of the secretion, namely, that the contents of the stomach were acid.

It is obvious from the foregoing statements that the results of the experiments of Edinger and Fränkel with sodium alizarin and sodium rosanilin sulphonate were not in accord with what had been previously determined concerning the place of formation of hydrochloric acid in the stomach, inasmuch as they indicated the formation of acid in the pyloric mucous membrane which had previously been shown by Heidenhain ('70) and Klemensiewicz ('75) to secrete an alkaline fluid. Moreover, neither of these experiments gave any clear indication of the ource of the hydrochloric acid.

It is also clear that in order to solve this problem by the use of a chemical indicator the substance employed, in addition to being an indicator of acidity or alkalinity, must have the properties of a vital stain, that is to say, the cells of the gastric glands must be freely permeable to it and it must have a special affinity for constituents of the gastric secretion in the glands, or the distribution coefficient must favor its concentration in this secretion in sufficient amounts to give a distinct color reaction. These conditions we have found to be fulfilled by neutral red and by a number of dyes belonging to the naphtol blue series including nile blue, and the various cyanamins discovered by Witt ('90).

Our first successful experiments in staining specifically the secretion in the parietal cells and in the lumina of the gastric glands were obtained with Grübler's napthalin blue R crystals (a trade name for naphtol blue). Solutions of this dye in normal salt solution, injected into the blood vessels of the recently killed animal, were found to stain the secretion in the canaliculi of the parietal cells and in the lumina of the gland tubules of the fundus region a distinct red color, while the cells of the foveola and the mucus on the free surface were stained a deep blue.

Tests of the solution of naphtalin blue afforded no explanation of this result, inasmuch as addition of acid produced no change in the color of the solution, and addition of sodium hydrate gave a green color. Accordingly, it seemed probable that the reaction observed was due either to another dye present in the naphtalin blue as an impurity, or to a new dye synthesized during the process of staining.

After consideration of the commercial process for the manufacture of the naphtol blues it seemed probable, in view of the fact that dimethylparaphenylendiamin is a biproduct of the synthesis of naphtol blue from nitrosodimethylanilin and B naphtol, that the dye on which this reaction depended would prove to be cyanamin, which, according to Witt, is formed when the mixture of naphtol blue and dimethylparaphenylendiamin resulting from the synthesis above mentioned is boiled for a time with an alcoholic solution of potassium hydroxide. Accordingly, cyanamin chloride was prepared by the process described by Witt, and its solutions tested on the gastric mucous membrane.

Cyanamin, according to Witt, possesses two basic groups, one molecule of the base combining with two molecules of hydrochloric acid to form a bichloride which is soluble in water with a deep blue color. On dilution of this solution the compound is broken up into a monochloride insoluble in water, which deposits as a reddish violet precipitate, and hydrochloric acid, which remains in solution. On the addition of alkalies the solution changes to a red color and after a short time the base settles out as a red flocculent precipitate.

On account of the formation of a monochloride intermediate in color between the red base and the blue bichloride it is apparent that as an indicator of reaction cyanamin does not approach in delicacy of response the more commonly used chemical indicators. But when we consider the relatively high content of hydrochloric acid in the gastric juice (as high as .5822 per cent. according to Rosemann ('07)) this is of little importance, for we have found that in dilute solutions of the dye a concentration of .0009 per cent. of hydrochloric acid, or approximately 1/600 of the concentration in the gastric juice, is sufficient to abolish all trace of red color. Furthermore, if Pawlow's idea is true that the native secretion has a constant acidity, and that the variations in acidity of the secretion from a gastric fistula are due to different degrees of neutralisation by the alkaline mucous secretion of the surface epithelium, then we might expect a maximum acidity in the gland lumen assuming that the hydrochloric acid is secreted as such by the cells. It follows therefore that if cyanamin stains the gastric secretion in the glands it will stain it blue wherever the acid is produced.

The method of applying the cyanamin is as follows: A fresh concentrated solution of the bichloride in normal sodium chloride solution is prepared; the animal is killed by a blow on the head, or by bleeding from the carotid, and the stomach exposed as rapidly as possible; a small piece of the mucous membrane is cut out with scissors, rinsed in normal salt solution, and placed in the solution of the dye. A few minutes' immersion suffices to accomplish the staining. When this is complete the piece of mucous membrane is placed on a slide with the mucous surface downwards and observed with a low power of the microscope.

If the staining has progressed far enough the edge of the preparation may be teased with needles and the superficial glands which alone are stained so isolated, when a cover glass is applied and the preparation studied by high power objectives. For these experiments we have used rabbits, guinea pigs, cats, and dogs.

In such preparations certain cells scattered throughout the glands promptly stain blue, the blue color affecting not only the protoplasm but the nucleus. These belong to both classes of cells constituting the glands and are interpreted by us as dead cells. In addition the small cells, first described by R. Heidenhain, which occur in small numbers scattered among the other epithelial cells of the gland, and the nature of which is still obscure, stain blue, but in this case the blue stain is confined to the granules with which the protoplasm of these cells is studded, the nucleus remaining unstained. Certain glands on the very edge of the preparation may stain bluish red, these being for the most part glands which have been actually injured in making the preparations.

In the uninjured glands reached by the dye, on the contrary, a uniform and characteristic reaction is obtained. With the exception of the dead cells and the small cells of Heidenhain mentioned above, the dye is entirely confined to the secretion in the lumina of the glands and their various diverticula, including the whole basketwork of canaliculi in the parietal cells—all of which was intensely stained. Moreover, in no place in this system of gland tubules below the level of the gastric foveolæ was the blue color of the acid solutions of the dye obtained. On the contrary the secretion contained in the canaliculi of the parietal cells was a distinct red like that displayed by the dye in alkaline solutions, while the secretion in the lumen of the gland was a bluish red. The short canaliculi connecting the parietal cell system of intracellular channels with the main lumen of the gland showed a color shading from the red of the content of the latter to the bluish red of the contents of the gland lumen. At the level of the bottoms of the foveolæ the color of the secretion changed rapidly to the pure blue of the acid solutions of cyanamin, and the cylindrical cells of the surface and of the foveolæ stained the acid color also.

Inasmuch as the results just described indicated that in no part of the gland system below the foveolar level did the secretion of the gastric gland cells have an acid reaction, and that the secretory contents of the parietal cells were even alkaline in reaction it was important to test the behavior in different states of physiological activity of these glands towards solutions of the dye. These experiments were performed on dogs, animals being kept without food for twenty-four hours, and compared, as regards the reaction with cyanamin chloride, with other animals at different intervals after feeding. These experiments showed that the resting gastric glands gave no reaction with cyanamin, while glands taken from active stomachs fifteen or more minutes after secretion gave the pronounced and characteristic reaction described above. Accordingly, the alkaline reaction of the contents of the canaliculi of the parietal cells, and the non-acid reaction of the contents of the lumen of the gland proper, are not the reactions of resting glands, but only of active glands from a stomach which is forming an acid secretion.

The amount of cyanamin chloride at our disposal did not permit our testing its action on the stomach when injected into the living animal intravenously, or by injection immediately after death of solutions through the blood vessels. Naphtol blue, however, apparently owes its properties in this connection to admixture of cyanamin, or to synthesis of the latter during the process of staining, for the reaction which it gives is exactly that of the pure cyanamin solutions, and we have been able to separate from the commercial zinc naphtol blue double chloride small quantities of cyanamin. Naphtol blue dissolved in normal salt solution injected from the aorta in a rabbit killed shortly after feeding will produce this reaction in every gland of the fundus region of the stomach. It is difficult, however, by this method, to secure a staining of the entire gland, for reasons which a consideration of the blood supply of the mucous membrane will make apparent. The bases of the glands stain well, but it is difficult to secure a staining of the upper portions of the glands. Preparations made in this way give, however, the most remarkable demonstration of the canalicular system of the glands that we have ever seen, resembling except for the color a perfect silver chromate impreg-

nation of this system. We have also obtained a feeble reaction by means of intravenous injection of solutions of naphtol blue.

Confirmatory Tests.

In view of the results obtained with cyanamin chloride it seemed probable that other dyes closely related to this substance would give similar reactions, and accordingly we prepared by acting on naphtol blue with anilin, according to the method described by Nietzki and Bossi ('92), the closely related dye named by the former phenylated nile blue. Solutions of this dye gave by far the most striking results obtained inasmuch as the intensely red base was precipitated in the canaliculi of the parietal cell, while the secretion in the gland lumen was stained a bluish red color. Similar results were obtained with solutions of nile blue sulphate, but a less pronounced reaction was obtained, the content of the parietal cells staining in this case bluish red, that of the lumina of the glands blue.

Neutral red, which has been highly commended by Ehrlich as an indicator for biological studies, next suggested itself in this connection for we had long known that it stained the secretion in the gastric glands and in the parietal cells. This dye may be used like cyanamin by immersing the fresh mucous membrane in a 1 in 10,000 solution in normal salt solution or by injecting such a solution of the dye through the blood vessels. In neutral solutions neutral red possesses a reddish color with a suggestion of orange. Alkaline solutions precipitate the base in the form of a yellow precipitate while acid solutions produce a crimson color. This dye therefore is capable of indicating either acidity, alkalinity or neutrality. In preparations made as indicated above of the fresh actively secreting fundus mucous membrane of the stomach neutral red promptly stains the secretion in the canaliculi of the parietal cells and in the main lumen of the gland. In the parietal cells the color assumed is the unmistakable yellow of the free base, in the lumen of the gland the color approaches more closely to the neutral tint, while the short diverticula of the lumina which connect the parietal cell with the lumen are of an intermediate tint. The whole system, however, is without question on the alkaline side of the reaction with

neutral red. At the bottom of the foveolæ the alkaline reaction gives way to the crimson acid color which is exhibited by the whole foveola and by the surface. The foveolar epithelium also stains the crimson acid tint. Neutral red stains the dead cells deep red, and also the granules of the small cells of Heidenhain referred to above.

Thus the consistent results of four separate methods show that the hydrochloric acid is not free as such in the gland, and that the contents of the canaliculi of the parietal cell contrary to expectation are alkaline in reaction. The question naturally arises, then, where is the acid of the gastric juice formed and what are the factors concerned in its formation? Without doubt, our reactions with the dyes of the cyanamin series indicate that the hydrochloric acid of the gastric juice is set free in the foveola, possibly also on the free surface of the mucous membrane. As to the source of the chlorine concerned in the formation of hydrochloric acid of the stomach the experiments of Greenwood ('85), Macallum ('08), and Fitzgerald ('10) seem to be conclusive. Greenwood showed that in preparations of the mucous membrane of the stomach made with silver nitrate, and then reduced in the light, the parietal cells stained much more strongly with the silver deposit than the other epithelial elements. In his studies of the silver reaction for chlorides Macallum showed that only chlorides, phosphates, and carbonates, of silver gave this reduction reaction, and devised a method by means of which the phosphates and carbonates could be excluded and only chlorides exhibited. This method consisted in using for the reaction a solution of silver nitrate containing nitric acid in which the phosphates and carbonate of silver are soluble. By this means he demonstrated that the parietal cells of the stomach were rich in chlorides. This result has recently been confirmed by Miss Fitzgerald, who found that the reaction was obtained not only in the body of the parietal cell but also in the intracellular channels.

This being the case, in view of the fact that the secretion of the parietal cells is alkaline while in the cells themselves, and that the secretion of the whole gland while contained in the gland lumen is very nearly neutral as shown by the neutral red and cyanamin reactions, it seems probable that the chlorine is

secreted by the parietal cells in the form of a chloride of an organic base, and that the hydrochloric acid is only set free after this secretion is poured out of the gland into the foveola. As to the nature of this base, there are some facts which suggest the probability that it is protein in nature. Stöhr's ('82) description of the parietal cells in man indicates clearly that he perceived a coagulated substance in the canals which connect these cells with the lumen of the gland, and Revell has succeeded in staining the content of the intracellular canals of the parietal cells with carmin solutions in material fixed in an alcohol bichromate sublimate mixture.

THE CONSISTENCE OF THE SECRETION IN THE GLAND LUMEN.

The fact that we were able to stain the secretion of the gastric glands while still contained in the gland has enabled us to study certain properties of this secretion. In the actively secreting rabbit stomach the lumen of the gland is widened by the accumulation of the secretion, and, by pressure on the cover glass, or by teasing, it is possible to expel the secretion from the gland, or to liberate it in the salt solution used for mounting and thus to learn something about the change in concentration of the secretion which takes place as it proceeds towards the surface of the mucous membrane. The assumption which is generally made that the secretion is formed by the glands in the same concentration as it presents when it emerges from the openings of the foveolæ, would lead one to suppose that the secretion in the gland would be a limpid solution, which would flow easily from the gland, and would mix readily with salt solution. This, however, proved not to be the case. When water from the surrounding salt solution enters the gland lumen the column of secretion breaks up into round droplets which maintain their individuality for several minutes. Similarly, when secretion is expressed from the gland lumen into the surrounding solution it collects around the mouth of the gland in large spherical droplets which slowly dissolve, the red reaction also at the same time slowly changing to the blue acid reaction, if the secretion has been stained with cyanamin. From these observations we are obliged to conclude that the secretion formed in the gland possesses a

relatively high content of solids, and that the bulk of the water found in the gastric secretion is added at the level of the glandular foveolæ.

OTHER CONSIDERATIONS.

Since it is apparent that the contents of the gastric glands proper when in a state of normal activity are not acid in reaction, and may even be alkaline, it follows that the ferment of the same secretion in the gland lumen is probably not in an active form, since, as is well known, pepsin is destroyed by alkalies while pepsinogen is not affected. Hence, the failure of the secretion to attack the cells themselves requires no further explanation in the case of the gastric glands than in the case of the pancreas, since in neither case does the activated ferment come in immediate contact with the parenchyma cells. This being the case it is pertinent to enquire whether under any conditions the secretion within the gland may become acid in reaction, for, in this event it is probable that the ferment would be activated and as happens under similar conditions in the pancreas the adjacent parenchyma cells would be attacked. This possibility is suggested by certain results obtained with the Claude Bernard reaction, where a reaction was obtained in the neighborhood of recent injuries to the mucous membrane far down the lumen of the gland, though the rest of the mucous membrane showed no reaction in the glands. We have as yet not had the opportunity to test this question by means of the cyanamin and neutral red reactions, but hope to report on this matter in the near future.

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